

AMENDMENTS TO THE CLAIMS

The following listing of claims will replace all prior versions and listings of claims in the application.

1-235. (cancelled)

236. (currently amended) A method of producing a heterologous polypeptide, the method comprising:

a) providing a transgenic plant or a transgenic plant cell comprising a recombinant DNA molecule, the recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,

i) a plant promoter;

[[i]]ii) a sequence complementary to a coding sequence for a heterologous polypeptide;

[[ii]]iii) a sequence complementary to a plant virus internal ribosome entry site; and

[[iii]]iv) a 3' UTR sequence of a first positive strand single-stranded RNA plant virus having a sequence encoding a viral RNA replication initiation site;

b) growing the transgenic plant or transgenic plant cell; and

c) producing an RNA transcript of the DNA sequence in the transgenic plant or the transgenic plant cell, the RNA transcript being a complementary RNA copy of the DNA sequence;

[[c]]d) providing a stimulus to the transgenic plant or transgenic plant cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA infecting the transgenic plant or the transgenic plant cell with an RNA nucleic acid operable to recognize the viral RNA replication initiation site and convert the RNA transcript produced by the transgenic plant or the transgenic plant cell to a translatable mRNA, the mRNA having a RNA sequence comprising, in the 5' to 3' direction:

i) a sequence complementary to the 3' UTR sequence;

ii) a coding sequence of the plant virus internal ribosome entry site; and

iii) a coding sequence of the heterologous polypeptide; and

e) translating the translatable mRNA in the transgenic plant or the transgenic plant cell to form the heterologous polypeptide.

237. (cancelled)

238. (currently amended) The method of producing a heterologous polypeptide of claim 236, wherein the plant promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

239. (currently amended) The method of producing a heterologous polypeptide of claim ~~236~~238, wherein the ~~eukaryotic~~-constitutive promoter is a cauliflower mosaic virus 35S promoter.

240. (currently amended) The method of producing a heterologous polypeptide of claim 236, wherein the coding sequence for ~~[[a]]~~the heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

241. (previously presented) The method of producing a heterologous polypeptide of claim 236, wherein the internal ribosome entry site is selected from the group consisting of a turnip mosaic potyvirus IRES, a tobamovirus IRES, and a hibiscus chlorotic ringspot virus IRES.

242. (withdrawn) The method of producing a heterologous polypeptide of claim 236, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

243. (currently amended) The method of producing a heterologous polypeptide of claim 236, wherein the 3' UTR ~~of a first positive strand single-stranded RNA plant virus~~ is a 3' UTR of a first sequence is obtained from a positive strand single-stranded RNA plant virus RNA with having no DNA stage.

244. (previously presented) The method of producing a heterologous polypeptide of claim 236, further comprising a sequence complementary to an intron.

245. (currently amended) The method of producing a heterologous polypeptide of claim 236, wherein said DNA ~~molecule~~ sequence further comprises a transcription termination signal sequence.

246. (previously presented) The method of producing a heterologous polypeptide of claim 236, wherein the transgenic plant is a dicotyledonous plant.

247. (previously presented) The method of producing a heterologous polypeptide of claim 246, wherein the dicotyledonous plant is a *Nicotiana* plant.

248. (previously presented) The method of producing a heterologous polypeptide of claim 247, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

249. (currently amended) The method of producing a heterologous polypeptide of claim 236, wherein the ~~providing a stimulus to infecting~~ the transgenic plant or ~~the~~ transgenic plant cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises infecting the transgenic plant or ~~the~~ transgenic plant cell with a ~~second~~ positive strand single-stranded RNA plant virus operable to recognize the viral RNA replication initiation site and convert the RNA transcript produced by the transgenic plant or the transgenic plant cell to a translatable mRNA.

250. (currently amended) The method of producing a heterologous polypeptide of claim 249, wherein the ~~second~~-positive strand single-stranded RNA plant virus is a ~~second~~-positive strand single-stranded RNA plant virus having no DNA stage.

251. (currently amended) The method of producing a heterologous polypeptide ~~in a transgenic plant or transgenic plant cell~~ of claim 250, wherein the ~~second~~-positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a ~~second~~ Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

252-256. (cancelled)

257. (currently amended) The method of producing a heterologous polypeptide of claim 236, wherein the ~~RNA comprising at least one sequence encoding a polypeptide component of an RNA virus replication complex is an~~ RNA comprising a nucleic acid comprises a polynucleotide sequence encoding an RNA-dependent RNA polymerase.

258. (currently amended) The method of producing a heterologous polypeptide of claim 236, wherein the ~~molar concentration ratio of heterologous polypeptide produced in a cell provided a stimulus relative to~~ infected with the RNA nucleic acid when compared as a molar ratio to the amount of the heterologous polypeptide produced in a cell not provided a stimulus is the RNA nucleic acid, ranges at least from about 50:1 to about 10,000:1.

259. (withdrawn) The method of producing a heterologous polypeptide of claim 236, wherein said method of producing a heterologous polypeptide in a transgenic plant is used to confer disease resistance to a transgenic plant further comprising conferring resistance to subsequent infection from a second positive strand single-stranded RNA virus.

260. (currently amended) A recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction:

a) a plant promoter;

[[a]]b) a sequence complementary to a coding sequence for a heterologous polypeptide;

[[b]]c) a sequence complementary to a plant internal ribosome entry site;

and

[[c]]d) a 3' UTR sequence comprising a DNA sequence of a 3'UTR RNA sequence of a positive strand single-stranded RNA plant virus.

261. (currently amended) The recombinant DNA molecule of claim 260, wherein the plant promoter is [[a]] selected from the group consisting of a constitutive promoter and an inducible promoter.

262. (currently amended) The recombinant DNA molecule of claim ~~260~~261, wherein the ~~eukaryotic~~ constitutive promoter is a cauliflower mosaic virus 35S promoter.

263. (currently amended) The recombinant DNA molecule of claim 260, wherein the coding sequence for [[a]]the heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

264. (currently amended) The recombinant DNA molecule of claim 260, wherein the sequence complementary to [[an]] a plant internal ribosome entry site is a sequence ~~complementary~~ complementary to an IRES a plant internal ribosome entry site (IRES) selected from the group consisting of a turnip mosaic potyvirus IRES, a tobamovirus IRES, and a hibiscus chlorotic ringspot virus IRES.

265. (currently amended) The recombinant DNA molecule of claim 260, wherein the sequence complementary to [[an]] a plant internal ribosome entry site is a sequence complementary to a tobamovirus internal ribosome entry site.

266. (currently amended) The recombinant DNA molecule of claim 260, wherein the 3' UTR of a ~~positive strand single-stranded RNA plant virus~~ is a 3' UTR of DNA sequence is a DNA copy of a positive strand single-stranded RNA plant virus RNA having no DNA stage.

267. (currently amended) The recombinant DNA molecule of claim 266, wherein the ~~3' UTR of a positive strand single-stranded RNA virus~~ RNA having no DNA stage is a 3' UTR of a bromovirus.

268. (previously presented) The recombinant DNA molecule of claim 260, further comprising a sequence complementary to an intron.

269. (previously presented) The recombinant DNA molecule of claim 260, further comprising a transcription termination signal.

270. (previously presented) A transgenic plant comprising the recombinant DNA molecule of claim 260.

271. (previously presented) A transgenic plant cell of claim 270.

272. (previously presented) The transgenic plant of claim 270, wherein the transgenic plant is a transgenic dicotyledonous plant.

273. (previously presented) The transgenic dicotyledonous plant of claim 272, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

274. (previously presented) Transgenic seed comprising the recombinant DNA molecule of claim 260.

275. (withdrawn) A vector having at least one site for insertion of a recombinant DNA construct for expressing a heterologous polypeptide in a transgenic cell of claim 260 comprising coding sequence of a heterologous polypeptide in an antisense orientation.

276. (withdrawn) A vector according to claim 275, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

277. (withdrawn) A vector according to claim 275, wherein the recombination site is selected from the group consisting of a bacteriophage lambda att site and a topoisomerase I-based recombination site.

278. (withdrawn) A vector according to claim 275, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

279. (withdrawn) A vector according to claim 275, wherein the at least one restriction enzyme recognition site comprises a polylinker.

280. (withdrawn) A recombinant RNA molecule comprising, in the 5' to 3' direction:

- a) an RNA sequence comprising a sequence complementary to a coding sequence for a heterologous polypeptide;
- b) a sequence complementary to an internal ribosome entry site; and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

281. (withdrawn) The recombinant RNA molecule of claim 280, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

282. (withdrawn) The recombinant RNA molecule of claim 280, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a *Leishmania* RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4G1 mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a *Rhopalosiphum padi* virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

283. (withdrawn) The recombinant RNA molecule of claim 280, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

284. (withdrawn) The recombinant RNA molecule of claim 280, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

285. (withdrawn) The recombinant RNA molecule of claim 284, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

286. (withdrawn) The recombinant RNA molecule of claim 280, further comprising a sequence complementary to an intron.

287. (withdrawn) A transgenic cell or transgenic plant comprising the recombinant RNA molecule of claim 280.

288. (withdrawn) The transgenic cell of claim 287, wherein the transgenic cell is a transgenic plant cell.

289. (withdrawn) The transgenic plant of claim 287, wherein the transgenic plant is a transgenic dicotyledonous plant.

290. (withdrawn) The transgenic dicotyledonous plant of claim 289, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

291. (withdrawn) The transgenic *Nicotiana* plant of claim 290, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

292. (withdrawn) An RNA complement of a recombinant RNA molecule, of claim 280 further comprising the complement comprising, in the 5' to 3' direction:

- a) a sequence complementary to a 3' UTR of a positive strand single-stranded RNA virus;
- b) an internal ribosome entry site; and
- c) an RNA sequence encoding a heterologous polypeptide.

293. (withdrawn) The RNA complement of a recombinant RNA molecule of claim 280, wherein the RNA sequence encoding a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

294. (withdrawn) A recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the recombinant DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to DNA sequence comprising:

- a) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;
- b) a sequence complementary to an internal ribosome entry site; and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

295. (withdrawn) The recombinant DNA molecule of claim 294, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

296. (withdrawn) The recombinant DNA molecule of claim 294, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NtIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein

mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

297. (withdrawn) The recombinant DNA molecule of claim 294, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

298. (withdrawn) The recombinant DNA molecule of claim 294, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES, a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4G1 mRNA IRES, a *Plautia stali* intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic

protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

299. (withdrawn) The recombinant DNA molecule of claim 294, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

300. (withdrawn) The recombinant DNA molecule of claim 294, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

301. (withdrawn) The recombinant DNA molecule of claim 294, further comprising a sequence complementary to an intron.

302. (withdrawn) The recombinant DNA molecule of claim 294, further comprising a transcription termination signal.

303. (withdrawn) The recombinant DNA molecule of claim 294, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

304. (withdrawn) The recombinant DNA molecule of claim 294, wherein the recombination site is selected from the group consisting of a bacteriophage lambda att site and a topoisomerase I-based recombination site.

305. (withdrawn) The recombinant DNA molecule of claim 294, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous

polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

306. (withdrawn) A kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell, the kit comprising a DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to a DNA sequence comprising:

- a) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;
- b) a sequence complementary to an internal ribosome entry site; and
- c) a 3' UTR of a positive strand single-stranded RNA virus.

307. (new) The method of producing a heterologous polypeptide of claim 236, wherein infecting the transgenic plant or the transgenic plant cell with an RNA nucleic acid comprises infecting the transgenic plant or the transgenic plant cell with a positive strand single-stranded RNA plant virus having a RNA genome operable to recognize and activate the viral RNA replication initiation site and convert the RNA transcript produced by the transgenic plant or transgenic plant cell to a translatable mRNA.

308. (new) The method of producing a heterologous polypeptide of claim 236, wherein infecting the transgenic plant or the transgenic plant cell with an RNA nucleic acid comprises transfecting the transgenic plant or the transgenic plant cell with a RNA of a positive strand single-stranded virus operable to recognize and activate the viral RNA replication initiation site and convert the RNA transcript produced by the transgenic plant or transgenic plant cell to a translatable mRNA.